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Design and synthesis of a new bicyclic dipeptide isostere

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The synthesis of a new Gly-Pro turn mimetic and the computational study of its ability to induce β-turn is reported.

In recent years several strategies have been adopted to limit the conformational space of peptide chains[1] and many efforts have been made to develop β-turn mimetics.[2]

It is known that a *cis* peptide moiety is geometrically suited for inducing the peptide chain to bend and that β-turns of type VI are characterized by a *cis*-Pro in the *i* + 2 position.[3] The control of the *cis*-prolyl amide geometry has been effectively achieved by tethering the α-carbon of the *N*-terminal amino acid residue to the proline 2-position.[4] Particularly, the bicyclic dipeptide analogues **1**, in which the α positions are joined by a two (X = $–CH_2CH_2–$) or three (X = $–CH_2NHCO–$) atom bridge, have been synthesized[4] to serve as mimetics of dipeptide *cis*-Xxx-Pro (Fig. 1). To reduce the conformational freedom of dipeptide mimetics like **1**, a shorter link could be introduced. In this way the pyrrolizidine amino acids **1** (X = $–CH_2–$), containing the *cis*-Xxx-Pro residue, would result (Fig. 1).

In this communication we report the synthesis of the GPTM (Gly-Pro Turn Mimetic) **2** (Fig. 1) either in racemic or enantiomerically pure form and its successful coupling with L-Boc-Phe-OH.

The 1,3-dipolar cycloaddition (1,3-DC) of nitrone **3**[5] and an acrylic acid derivative, followed by reductive opening of the isoxazolidine ring and intramolecular cyclization was envisaged as a rapid route to afford the bicyclic lactam **9**.

The 1,3-DC reaction was performed under different conditions and the best results were obtained from **3** and acrylamide (**4**) in water at 60 °C (Scheme 1) which afforded the 2-aminocarbonylhexahydropyrrolo[1,2-*b*]isoxazoles **5** and **6** and their 3-aminocarbonyl isomers **7** in 4 : 1 ratio and excellent yield (98%). Although the *endo–exo* selectivity of the cycloaddition was very low (6% de) this approach was synthetically convenient because both the diastereomeric adducts **5** and **6** could be transformed into **9**.

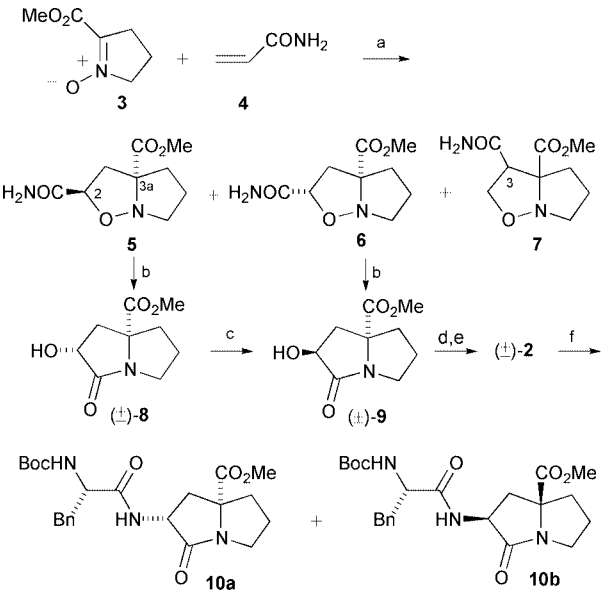
The pyrrolizidine **9** was obtained from **6** through the domino process N–O bond hydrogenolysis/intramolecular trans-amidation. Moreover, the *cis* substituted pyrrolizidine **8**, analogously derived from **5**, could be quantitatively isomerized to the corresponding *trans* hydroxy ester **9** by treatment with NaOH–MeOH at 70 °C followed by methylation with CH_2N_2. Therefore, the intermediate **9**, possessing the bicyclic skeleton of the GPTM **2**, was achieved in 55% overall yield starting from **3** and **4** (Scheme 1).

The *trans* alcohol **9** was easily transformed into the corresponding *cis* amine **2** by mesylation followed by a nucleophilic displacement with NaN_3 and reduction of the azido group with Ni-Raney (Scheme 1). The lactam **2**, was immediately coupled with an amino acid such as Boc-L-Phe, to test the reactivity of the amino group towards the peptide synthesis and to avoid the possible epimerization to the thermodynamically more stable *trans* isomer. The two diastereomeric tripeptides **10a** and **10b** were separated by chromatography on silica gel and fully characterized.

To assign the absolute configuration of **10a** and **10b**, a sample of the racemic *cis* alcohol **8** was resolved through the formation of the corresponding diastereomeric esters of *R*-Mosher acid, and one of the separated esters was analyzed by single crystal X-ray crystallography.† Consequently, both the diastereomeric esters were assigned their absolute configuration. After hydrolysis and treatment with CH_2N_2 the enantiomerically pure alcohols (2*R*,7a*R*)-**8** and (2*S*,7a*S*)-**8** were obtained, and transformed into **10a** and **10b**, respectively, through the previously described procedure.

The investigation of the turn-inducing potential of both the enantiomers (2*R*,7a*R*)-**2** and (2*S*,7a*S*)-**2** has been carried out through a simulation procedure run on model hexapeptides according to the criteria recently proposed by Müller *et al.*[6]

The model hexapeptides Ac-Ala-Ala-GPTM-Ala-Ala-NHMe **11** and **12** (Fig. 2), containing in the central position *RR*-GPTM and *SS*-GPTM respectively, were examined for their conformational freedom by Monte Carlo (global search



Scheme 1 a: H_2O, 60 °C, 14 h (**5**: 37%, **6:** 42%, **7**: 19%). b: $Pd(OH)_2$ (cat), MeOH, AcOH, H_2, 12 h (98%). c: (i) NaOH, MeOH, 70 °C, 2 h; (ii) Dowex 50; (iii) CH_2N_2; (60%). d: (i) MsCl, py; (ii) NaN_3, DMF; (87%). e: Ni-Raney (80%). f: L-Boc-Phe-OH, PyBroP, DiPEA, CH_2Cl_2 (55%).

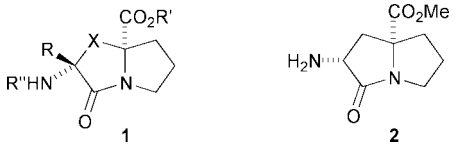


Fig. 1 Mimetics of *cis*-Xxx-Pro dipeptide and Gly-Pro turn mimetic.

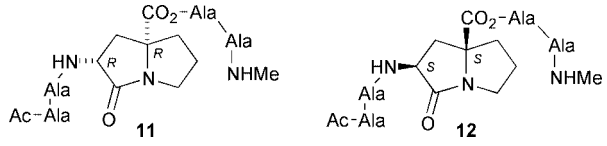


Fig. 2 Model hexapeptides.

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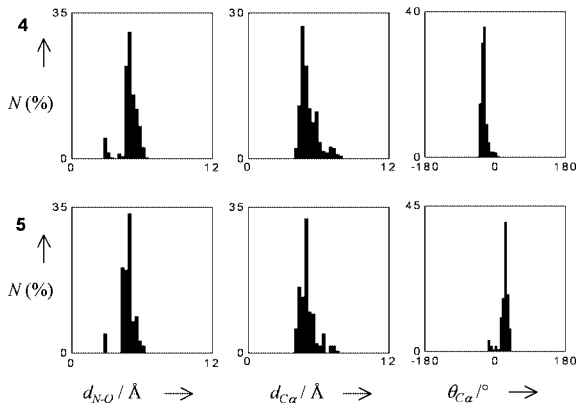

Fig. 3 Percentage distribution N of $d_{N\text{-}O}$, $d_{C\alpha}$ and $\theta_{C\alpha}$ values in the conformers of **4** and **5** within 6 kcal mol^{-1} of the global minimum. (Each column spans 0.3 Å or 6°.)

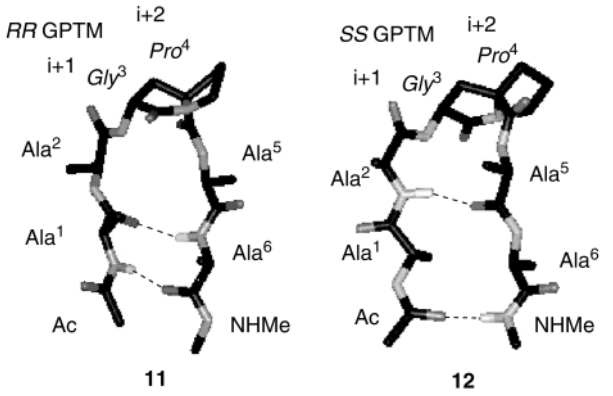

Fig. 4 Structures of two low energy conformers of **11** and **12**. Legend: C: black, N: pale grey, O: grey, H: white. For reasons of clarity only the H atoms involved in hydrogen bondings (--) were depicted.

MCMM) procedure.[7] The following parameters were used to establish the presence of a reverse turn: the donor–acceptor NH_{Ala5}–CO_{Ala2} distance $d_{N\text{-}O}$, the $C\alpha_{Ala2}$–$C\alpha_{Ala5}$ distance $d_{C\alpha}$ and the virtual torsion angle $\theta_{C\alpha}$ (defined by $C\alpha_{Ala2}$–$C\alpha_{Gly3}$–$C\alpha_{Pro4}$–$C\alpha_{Ala5}$).[3,6] The percentage distribution of $d_{N\text{-}O}$, $d_{C\alpha}$ and $\theta_{C\alpha}$ values in the calculated conformations within 6 kcal mol^{-1} of the global minimum of **11** and **12** (318 and 170 conformers respectively) were reported in Fig. 3. The histograms showed a substantial restriction of the occupied conformational space of hexapeptides incorporating GPTMs **2**. Moreover, a very good portion of conformers possessed the $d_{C\alpha}$ and $\theta_{C\alpha}$ values characteristic of β-turn. In particular the percentages of conformers of **11** and **12** with $d_{C\alpha}$ less than 5 Å (one definition of a tight β-turn) were 55 and 58%, respectively, while almost all structures showed $d_{C\alpha}$ less than 7 Å (**11**: 94%, **12**: 96%).[3] All conformers had $|\theta_{C\alpha}|$ under 50°, and $|\theta_{C\alpha}|$ under 30° present in 54 and 75% conformers of **11** and **12**, respectively.

On the contrary, the presence of the hydrogen bonding characteristic of classical β-turn ($d_{N\text{-}O}$ < 3.5 Å) was found in a small fraction of conformers (**11**: 7%; **12**: 5%). However, the intramolecular hydrogen bond was not found critical for the stability of a β-turn,[3] and seems not to be necessary in peptides incorporating mimics **2**, because the three torsion angles (ψ_{Gly3}, ω_{Pro4} and φ_{Pro4}) embedded in the 5,5-bicyclic structure and the spatial orientation of the terminal amino and carboxylic groups (on the same face of the bicyclic ring system) force the peptide chain to fold back upon itself.

The whole set of computational data clearly showed that bicyclic lactams like (2*R*,7a*R*)-**2** and (2*S*,7a*S*)-**2** were effective turn restraints when incorporated in the hexapeptides **11** and **12**.

The spatial arrangement of side chains is generally critical to recognition and bioactivity of peptides and its control is one of the goals of peptidomimetics. GPTMs (2*R*,7a*R*)-**2** and (2*S*,7a*S*)-**2** were shown to promote complementary relative orientation of the side chains of the residues near the reverse-turn.

As shown in Fig. 3, the $\theta_{C\alpha}$ values were prevalently negative in the conformers of **11**, but positive in those of **12** because of the enantiomeric relationship between the incorporated GPTMs. The presence of opposite reverse turns resulted also in an opposite orientation of the amino acid side chains as illustrated by a structural comparison of two representative low energy conformers of **11** and **12** (Fig. 4).

Both the selected structures were characterized by the presence of two intramolecular hydrogen bonds which indicated the initiation of an antiparallel β-sheet interaction between the two half-strands. In **11** the β-sheet hydrogen bonds were contiguous (between NH_{Ala6}–CO_{Ala1} and NH_{Ala1}–CO_{Ala6}) while in **12** were more distant (between NH_{Ala2}–CO_{Ala5} and NH_{NHMe}–CO_{Ac}). In **11** the methyl groups of Ala^2 and Ala^5 were situated under the back-bone plane (when the peptide chain was oriented as shown in Fig. 4) and those of Ala^1 and Ala^6 over the plane, while in **12** the opposite orientations occurred.

In conclusion both the enantiomers of GPTMs **2** were shown to be potentially useful reverse turn mimics. Attractive features of these new dipeptide surrogates were the reduced flexibility compared to analogous bicyclic systems, the complementary behavior of the two enantiomers in controlling the side-chain orientation, the rapid access to these systems starting from easily available compounds, the possibility of extending the process to the synthesis of other Xxx-Pro analogues (XPTMs) through the cycloaddition of nitrone **3** to 2-substituted acrylic acid derivatives.

Some structural modifications of GPTMs **2** for their use in solid phase syntheses and their incorporation into selected bioactive peptides for structure–activity relationship studies are currently under investigation in our laboratories.

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Notes and references

† *Crystallographic data* for (2*S*,7a*S*)-3-oxo-2-[(2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropionyloxy]tetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylic acid methyl ester: $C_{19}H_{20}F_3NO_6$, M = 415.36, orthorhombic, a = 8.2726(3), b = 11.9643(6), c = 19.746(2) Å, U = 1954.7(2) Å^3, T = 293 K, space group $P2_12_12_1$, Z = 4, μ(Cu-K$_\alpha$) = 1.067 mm^{-1}, 2168 reflections collected, 1992 independent (R_{int} = 0.0318) which were used in all calculations. The final $R1$ was 0.0399 and $wR2$ 0.1177 (all data).

CCDC 166488. See http://www.rsc.org/suppdata/cc/b1/b101692j/ for crystallographic data in .cif or other electronic format.

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